CLAIMS

1. A method of constructing a variant of a parent Termamyl-like α-amylase, which variant has α-amylase activity and at least one altered property as compared to said parent α-amylase, which method comprises

i) analysing the structure of the parent Termamyl-like α-amylase to identify at least one amino acid residue or at least one structural part of the Termamyl-like α-amylase structure, which amino acid residue or structural part is believed to be of relevance for altering said property of the parent Termamyl-like α-amylase (as evaluated on the basis of structural or functional considerations),

ii) constructing a Termamyl-like α-amylase variant, which as compared to the parent Termamyl-like α-amylase, has been modified in the amino acid residue or structural part identified in i) so as to alter said property, and, optionally,

iii) testing the resulting Termamyl-like α-amylase variant with respect to said property or properties.

2. The method according to claim 1, wherein the property to be altered is selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern, temperature stability, pH dependent activity, pH dependent stability (especially increased stability at low (e.g. pH<6) or high (e.g. pH>9) pH values), stability towards oxidation, $Ca^{2+}$-dependency and specific activity.

3. The method according to claim 1, wherein the property to be altered is the calcium ion dependency and the structural part to be modified is selected from the group consisting of the C domain, the interface between the A and B domain, the interface between the A and C domain, or the interaction to a calcium binding site of the Termamyl-like α-amylase.

4. The method according to claim 1, wherein the property to be altered is the substrate cleavage pattern and the structural part to be modified is located within 10Å from an amino acid residue of the substrate binding site.

5. A method of constructing a variant of a parent Termamyl-like α-amylase, which variant has α-amylase activity and one or more altered properties as compared to said parent α-amylase, which method comprises

i) comparing the three-dimensional structure of the Termamyl-like α-amylase with the structure of a non-Termamyl-like α-amylase,

ii) identifying a part of the Termamyl-like α-amylase structure which is different from the non-Termamyl-like α-amylase structure and which from structural or functional considerations is contemplated to be responsible for differences in one or more properties of the Termamyl-like and non-Termamyl-like α-amylase,

iii) modifying the part of the Termamyl-like α-amylase identified in ii) whereby a Termamyl-like α-amylase variant is obtained, one or more properties of which differ from the parent Termamyl-like α-amylase, and optionally,

iv) testing the resulting Termamyl-like α-amylase variant with respect to said property or properties.

6. The method according to claim 5, wherein, in step iii), the part of the Termamyl-like α-amylase is modified so as to resemble the corresponding part of the non-Termamyl-like α-amylase.

7. The method according to claim 5, wherein, in step iii), the modification is accomplished by deleting one or more amino acid residues of the part of the Termamyl-like α-amylase to be modified; by replacing one or more amino acid residues of the part of the Termamyl-like α-amylase to be modified with the amino acid residues occupying corresponding positions in the non-Termamyl-like α-amylase; or by insertion of one or more amino acid residues present in the non-Termamyl-like α-amylase into a corresponding position in the Termamyl-like α-amylase.

8. The method according to claim 5, wherein the non-Termamyl-like α-amylase structure is the structure of a fungal α-amylase or a mammalian α-amylase.

9. The method according to claim 8, wherein the non-Termamyl-like α-amylase is the *Aspergillus oryzae* TAKA α-amylase, the *A. niger* acid α-amylase, the *Bacillus subtilis* α-amylase or the pig pancreatic α-amylase.

10. The method according to claim 1, wherein the parent Termamyl-like α-amylase is derived from a strain of *Bacillus*.

11. The method according to claim 10, wherein the parent α-amylase is derived from a strain of a *B. licheniformis*, *B. amyloliquefaciens*, *B. stearothermophilus* or a strain from an alkalophilic *Bacillus* sp. such as NCIB 12289, NCIB 12512 or NCIB 12513.

12. The method according to claim 1, wherein the parent α-amylase is a hybrid α-amylase comprising a combination of partial amino acid sequences derived from at least two α-amylases, of which one is a Termamyl-like α-amylase and the other(s) are, e.g., from a microbial and/or a mammalian α-amylase.

13. The method according to claim 5, wherein the part of the parent Termamyl-like α-amylase to be modified and identified in step ii) is loop 1, loop 2, loop 3 and/or loop 8 of the parent α-amylase.

13. A method of constructing a variant of a parent Termamyl-like α-amylase, which has a decreased calcium ion dependency as compared to said parent, which method comprises:

i) identifying an amino acid residue within 10Å from a $Ca^{2+}$ binding site of a Termamyl-like α-amylase in a model of the three-dimensional structure of said α-amylase, which from structural or functional considerations is believed to be responsible for a non-optimal calcium ion interaction,

ii) constructing a variant in which said amino acid residue is replaced with another amino acid residue which from structural or functional considerations is believed to be important for establishing a higher $Ca^{2+}$ binding affinity, and

iii) testing the $Ca^{2+}$ dependency of the resulting Termamyl-like α-amylase variant.

14. A method of constructing a variant of a parent Termamyl-like α-amylase which variant has α-amylase activity and an altered pH dependent activity, which method comprises

i) in a three-dimensional structure of the Termamyl-like α-amylase in question, identifying an amino acid residue within 15Å from an active site residue, in particular 10Å from an active site residue, which amino acid residue is contemplated to be involved in electrostatic or hydrophobic interactions with an active site residue,

ii) replacing, in the structure, said amino acid residue with an amino acid residue which changes the electrostatic and/or hydrophobic surroundings of an active site residue and evaluating the accomodation of the amino acid residue in the structure,

iii) optionally repeating step i) and/or ii) until an amino acid replacement has been identified which is accomodated into the structure,

iv) constructing a Termamyl-like α-amylase variant resulting from steps i), ii) and optionally iii) and testing the pH dependent activity of said variant.

15. A method of increasing the thermostability and/or altering the temperature optimum of a parent Termamyl-like α-amylase, which method comprises

i) identifying an internal hole or a crevice of the parent Termamyl-like α-amylase in the three-dimensional structure of said α-amylase,

ii) replacing, in the structure, one or more amino acid residues in the neighbourhood of the hole or crevice identified in i) with another amino acid residue which from structural or functional considerations is believed to increase the hydrophobic interaction and to fill out or reduce the size of the hole or crevice,

iii) constructing a Termamyl-like α-amylase variant resulting from step ii) and testing the thermostability and/or temperature optimum of the variant.

16. A method of constructing a variant of a Termamyl-like α-amylase which has a reduced ability to cleave a substrate close to the branching point, which method comprises

i) identifying the substrate binding area of the parent Termamyl-like α-amylase in a model of the three-dimensional structure of said α-amylase,

ii) replacing, in the model, one or more amino acid residues of the substrate binding area of the cleft identified in i), which is/are believed to be responsible for the cleavage pattern of the parent α-amylase, with another amino acid residue which from

structural considerations is believed to result in an altered substrate cleavage pattern, or deleting one or more amino acid residues of the substrate binding area contemplated to introduce favourable interactions to the substrate or adding one or more amino acid residues to the substrate binding area contemplated to introduce favourable interactions to the substrate, and

iii) constructing a Termamyl-like α-amylase variant resulting from step ii) and testing the substrate cleavage pattern of the variant.

17. The method according to claim 1, in which the α-amylase variant is obtained by cultivating a microorganism comprising a DNA sequence encoding the variant under conditions which are conducive for producing the variant, and optionally subsequently recovering the variant from the resulting culture broth.

18. A variant of a parent Termamyl-like α-amylase, in which variant at least one amino acid residue of the parent α-amylase, which is/are present in a fragment corresponding to the amino acid fragment 44-57 of the amino acid sequence of SEQ ID NO: 4, has been deleted or replaced with one or more amino acid residues which is/are present in a fragment corresponding to the amino acid fragment 66-84 of the amino acid sequence shown in SEQ ID NO: 10, or in which one or more additional amino acid residues has been added using the relevant part of SEQ ID NO: 10 or a corresponding part of another Fungamyl-like α-amylase as a template.

19. A variant of a parent Termamyl-like α-amylase, which variant has a region which, when the amino acid sequence of variant is aligned most closely with the amino acid sequence of the said parent α-amylase, occupies the same position as the portion from residue X to residue Y of SEQ ID NO: 4, the said region having at least 80% sequence homology with the part of SEQ ID NO: 10 extending from residue Z to residue V of SEQ ID NO: 10, wherein

X is the amino acid residue occupying position 44, 45, 46, 47 or 48 of SEQ ID NO: 4,

Y is the amino acid residue occupying position 51, 52, 53, 54, 55, 56 or 57 of SEQ ID NO: 4,

Z is the amino acid residue occupying position 66, 67, 68, 69 or 70 of SEQ ID NO: 10, and

V is the amino acid residue occupying position 78, 79, 80, 81, 82, 83 or 84 of SEQ ID NO: 10.

20. The variant according to claim 18, wherein X is the amino acid residue occupying position 48 and Y the amino acid residue occupying position 51 of SEQ ID NO: 4 and Z is the amino acid residue occupying position 70 and V the amino acid residue occupying position 78 in SEQ ID NO: 10.

21. A variant of a parent Termamyl-like α-amylase, in which variant at least one of the amino acid residues of the parent α-amylase, which is/are present in an amino acid fragment corresponding to the amino acid fragment 195-202 of the amino acid sequence of SEQ ID NO: 4, has been deleted or replaced with one or more of the amino acid residues which is/are present in an amino acid fragment corresponding to the amino acid fragment 165-177 of the amino acid sequence shown in SEQ ID NO: 10, or in which one or more additional amino acid residues has been added using the relevant part of SEQ ID NO: 10 or a corresponding part of another Fungamyl-like α-amylase as a template.

22. A variant of a parent Termamyl-like α-amylase, which variant has a region which, when the amino acid sequence of variant is aligned most closely with the amino acid sequence of the said parent α-amylase, occupies the same position as the portion from residue X to residue Y of SEQ ID NO: 4, the said region having at least 80%, such as 90% sequence homology with the part of SEQ ID NO: 10 extending from residue Z to residue V of SEQ ID NO: 10, wherein

X is the amino acid occupying position 195 or 196 of SEQ ID NO: 4,

Y is the amino acid residue occupying position 198, 199, 200, 201, or 202 of SEQ ID NO: 4,

Z is the amino acid residue occupying position 165 or 166 of SEQ ID NO: 10, and

V is the amino acid residue occupying position 173, 174, 175, 176 or 177 of SEQ ID NO: 10.

23. The variant according to claim 21, in which the amino acid fragment of the parent α-amylase, which corresponds to amino acid residues 196-198 of SEQ ID NO: 4, has been replaced with the amino acid fragment corresponding to amino acid residues 166-173 of the amino acid sequence shown in SEQ ID NO: 10.

24. A variant of a parent Termamyl-like α-amylase, in which variant at least one of the amino acid residues of the parent α-amylase, which is/are present in a fragment corresponding to the amino acid fragment 117-185 of the amino acid sequence of SEQ ID NO: 4, has/have been deleted or replaced with one or more of the amino acid residues, which is/are present in an amino acid fragment corresponding to the amino acid fragment 98-210 of the amino acid sequence shown in SEQ ID NO: 10, or in which one or more additional amino acid residues has been added using the relevant part of SEQ ID NO: 10 or a corresponding part of another Fungamyl-like α-amylase as a template.

25. A variant of a parent Termamyl-like α-amylase, which variant has a region which, when the amino acid sequence of variant is aligned most closely with the amino acid sequence of the said parent α-amylase, occupies the same position as the portion from residue X to residue Y of SEQ ID NO: 4, the said region having at least 80%, such as at least 90% sequence homology with the part of SEQ ID NO: 10 extending from residue Z to residue V of SEQ ID NO: 10, wherein

X is the amino acid occupying position 117, 118, 119, 120 or 121 of SEQ ID NO: 4,

Y is the amino acid occupying position 181, 182, 183, 184 or 185 of SEQ ID NO: 4,

Z is the amino acid occupying position 98, 99, 100, 101, 102 of SEQ ID NO: 10, and

V is the amino acid occupying position 206, 207, 208, 209 or 210 of SEQ ID NO: 10.

26. The variant according to claim 24, in which an amino acid fragment of the parent α-amylase, which corresponds to amino acid residues 121-181 of SEQ ID NO: 4, has been

replaced with the amino acid fragment corresponding to amino acid residues 102-206 of the amino acid sequence shown in SEQ ID NO: 10.

27. A variant of a parent Termamyl-like α-amylase, in which variant at least one of the amino acid residues of the parent α-amylase, which is/are present in a fragment corresponding to the amino acid fragment 117-181 of the amino acid sequence of SEQ ID NO: 4, has/have been deleted or replaced with one or more of the amino acid residues, which is/are present in an amino acid fragment corresponding to the amino acid fragment to 98-206 of the amino acid sequence shown in SEQ ID NO: 10, or in which one or more additional amino acid residues has been added using the relevant part of SEQ ID NO: 10 or a corresponding part of another Fungamyl-like α-amylase as a template.

28. A variant of a parent Termamyl-like α-amylase, which variant has a region which, when the amino acid sequence of variant is aligned most closely with the amino acid sequence of the said parent α-amylase, occupies the same position as the portion from residue X to residue Y of SEQ ID NO: 4, the said region having at least 80%, such as at least 90% sequence homology with the part of SEQ ID NO: 10 extending from residue Z to residue V of SEQ ID NO: 10, wherein

X is the amino acid occupying position 117, 118, 119, 120 or 121 of SEQ ID NO: 4,

Y is the amino acid occupying position 174, 175, 176 or 177 of SEQ ID NO: 4,

Z is the amino acid occupying position 98, 99, 100, 101, 102 of SEQ ID NO: 10, and

V is the amino acid occupying position 199, 200, 201 or 202 of SEQ ID NO: 10.

29. The variant according to claim 27, in which the amino acid fragment of the parent α-amylase, which corresponds to amino acid residues 121-174 of SEQ ID NO: 4, has been replaced with the amino acid fragment corresponding to amino acid residues 102-199 of the amino acid sequence shown in SEQ ID NO: 10.

30. A variant of a parent Termamyl-like α-amylase, in which variant at least one of the amino acid residues of the parent α-amylase, which is/are present in an amino acid fragment corresponding to the amino acid fragment 12-19 of the amino acid sequence of SEQ ID NO: 4, has/have been deleted or replaced with one or more of the amino acid residues, which is/are present in an amino acid fragment which corresponds to the amino acid fragment 28-42 of SEQ ID NO: 10, or in which one or more additional amino acid residues has/have been inserted using the relevant part of SEQ ID NO: 10 or a corresponding part of another Fungamyl-like α-amylase as a template.

31. A variant of a parent Termamyl-like α-amylase, which variant has a region which, when the amino acid sequence of variant is aligned most closely with the amino acid sequence of the said parent α-amylase, occupies the same position as the portion from residue X to residue Y of SEQ ID NO: 4, the said region having at least 80%, such as at least 90% sequence homology with the part of SEQ ID NO: 10 extending from residue Z to residue V of SEQ ID NO: 10, wherein

X is the amino acid occupying position 12, 13 or 14 of SEQ ID NO: 4,

Y is the amino acid occupying position 15, 16, 17, 18 or 19 of SEQ ID NO: 4,

Z is the amino acid occupying position 28, 29, 30, 31 or 32 of SEQ ID NO: 10, and

V is an amino acid residue corresponding to the amino acid occupying position 38, 39, 40, 41 or 42 of SEQ ID NO: 10.

32. The variant according to claim 30, in which the amino acid fragment of the parent α-amylase, which corresponds to amino acid residues 14-15 of SEQ ID NO: 4, has been replaced with the amino acid fragment corresponding to amino acid residues 32-38 of the amino acid sequence shown in SEQ ID NO: 10.

33. A variant of a parent Termamyl-like α-amylase, in which variant at least one of the amino acid residues of the parent α-amylase, which is present in a fragment corresponding to amino acid residues 7-23 of the amino acid sequence of SEQ ID NO: 4, has/have been deleted or replaced with one or more amino acid residues, which is/are present in an amino

acid fragment corresponding to amino acid residues 13-45 of the amino acid sequence shown in SEQ ID NO: 10, or or in which one or more additional amino acid residues has/have been inserted using the relevant part of SEQ ID NO: 10 or a corresponding part of another Fungamyl-like α-amylase as a template.

34. A variant of a parent Termamyl-like α-amylase, which variant has a region which, when the amino acid sequence of variant is aligned most closely with the amino acid sequence of the said parent α-amylase, occupies the same position as the portion from residue X to residue Y of SEQ ID NO: 4, the said region having at least 80%, such as at least 90% sequence homology with the part of SEQ ID NO: 10 extending from residue Z to residue V of SEQ ID NO: 10, wherein

X is the amino acid occupying position 7 or 8 of SEQ ID NO: 4,

Y is the amino acid occupying position 18, 19, 20, 21, 22 or 23 of SEQ ID NO: 4,

Z is the amino acid occupying position 13 or 14 of SEQ ID NO: 10, and

V is the amino acid occupying position 40, 41, 42, 43, 44 or 45 of SEQ ID NO: 10.

35. The variant according to claim 33, in which the amino acid fragment of the parent α-amylase, which corresponds to amino acid residues 8-18 of SEQ ID NO: 4, has been replaced with the amino acid fragment corresponding to amino acid residues 14-40 of the amino acid sequence shown in SEQ ID NO: 10.

36. A variant of a parent Termamyl-like α-amylase, in which variant at least one of the amino acid residues of the parent α-amylase, which is present in a fragment corresponding to amino acid residues 322-346 of the amino acid sequence of SEQ ID NO: 2, has/have been deleted or replaced with one or more amino acid residues, which is/are present in an amino acid fragment corresponding to amino acid residues 291-313 of the amino acid sequence shown in SEQ ID NO: 10, or or in which one or more additional amino acid residues has/have been inserted using the relevant part of SEQ ID NO: 10 or a corresponding part of another Fungamyl-like α-amylase as a template.

37. A variant of a parent Termamyl-like α-amylase, which variant has a region which, when the amino acid sequence of variant is aligned most closely with the amino acid sequence of the said parent α-amylase, occupies the same position as the portion from residue X to residue Y of SEQ ID NO: 2, the said region having at least 80% sequence homology with the part of SEQ ID NO: 10 extending from residue Z to residue V of SEQ ID NO: 10, wherein

X is the amino acid occupying position 322, 323, 324 or 325 of SEQ ID NO: 2,

Y is the amino acid occupying position 343, 344, 345 or 346 of SEQ ID NO: 2,

Z is the amino acid occupying position 291, 292, 293 or 294 of SEQ ID NO: 10, and

V is the amino acid occupying position 310, 311, 312 or 313 of SEQ ID NO: 10.

38. The variant according to claim 36, in which the amino acid fragment of the parent α-amylase, which corresponds to amino acid residues 325-345 of SEQ D No. 2, has been replaced with the amino acid fragment corresponding to amino acid residues 294-313 of the amino acid sequence shown in SEQ ID NO: 10.

39. A variant of a parent Fungamyl-like α-amylase, in which variant at least one of the amino acid residues of the parent α-amylase, which is/are present in an amino acid fragment corresponding to amino acid residues 291-313 of the amino acid sequence of SEQ ID NO: 10, has/have been deleted or replaced with one or more of the amino acid residues, which is/are present in an amino acid fragment corresponding to amino acid residues 98-210 of the amino acid sequence shown in SEQ ID NO: 4, or in which one or more additional amino acid residues has/have been inserted using the relevant part of SEQ ID NO: 4 or a corresponding part of another Termamyl-like α-amylase as a template.

40. A variant of a parent Fungamyl-like α-amylase, which variant has a region which, when the amino acid sequence of variant is aligned most closely with the amino acid sequence of the said parent α-amylase, occupies the same position as the portion from residue X to residue Y of SEQ ID NO: 10, the said region having at least 80%, such as at least 90% sequence

homology with the part of SEQ ID NO: 10 extending from residue Z to residue V of SEQ ID NO: 4, wherein

X is the amino acid occupying position 117, 118, 119, 120 or 121 of SEQ ID NO: 10,

Y is the amino acid occupying position 181, 182, 183, 184 or 185 of SEQ ID NO: 10,

Z is the amino acid occupying position 98, 99, 100, 101 or 102 of SEQ ID NO: 4, and

V is the amino acid occupying position 206, 207, 208, 209 or 210 of SEQ ID NO: 4.

41. The variant according to claim 39, in which the amino acid fragment of the parent α-amylase, which corresponds to amino acid residues 121-181 of SEQ ID NO: 10, has been replaced with the amino acid fragment corresponding to amino acid residues 102-206 of the amino acid sequence shown in SEQ ID NO: 4.

42. A variant according to claim 39, in which the the amino acid fragment of the parent α-amylase, which corresponds to amino acid residues 121-174 of SEQ ID NO: 10, has been replaced with the amino acid fragment corresponding to amino acid residues 102-199 of the amino acid sequence shown in SEQ ID NO: 4.

43. A variant of a parent Fungamyl-like α-amylase, in which an amino acid fragment corresponding to amino acid residues 181-184 of the amino acid sequence shown in SEQ ID NO: 10 has been deleted.

44. A variant of a parent Termamyl-like α-amylase, which exhibits α-amylase activity and which has a decreased $Ca^{2+}$ dependency as compared to the parent α-amylase.

45. A variant according to claim 44, which comprises a mutation in a position corresponding to at least one of the following positions in SEQ ID NO 2:
N104, A349, I479, L346, I430, N457, K385, F350, I411, H408 or G303, in particular a mutation corrsponding to

N104D;
A349C+I479C;
L346C+I430C;
N457D,E;
N457D,E+K385R;
F350D,E+I430R,K;
F350D,E+I411R,K;
H408Q,E,N,D; and/or
G303N,D,Q,E.

46. A variant of a parent Termamyl-like α-amylase which exhibits a higher activity below the pH optimum than the parent α-amylase, which variant comprises a mutation of an amino acid residue corresponding to at least one of the following positions of the *B. licheniformis* α-amylase (SEQ ID NO: 2): E336, Q333, P331, I236, V102, A232, I103, L196, in particular at least one of the following mutations:
E336R,K;
Q333R,K; P331R,K;
V102R,K,A,T,S,G;
I236K,R,N;
I103K,R;
L196K,R; and/or
A232T,S,G.

47. A variant of a parent Termamyl-like α-amylase which exhibits a higher activity above the pH optimum than the parent α-amylase, which variant comprises a mutation of an amino acid residue corresponding to at least one of the following positions of the *B. licheniformis* α-amylase (SEQ ID NO: 2): N236, H281 and/or Y273, in particular one of the follwoing mutations:
N326I,Y,F,L,V;
H281F,I,L; and/or
Y273F,W.

48. A variant of a parent Termamyl-like α-amylase which exhibits α-amylase activity and which has an increased thermostability and/or altered temperature optimum as compared to the parent α-amylase, which variant comprises a mutation of an amino acid residue corresponding to at least one of the following positions of the *B. licheniformis* α-amylase (SEQ ID NO: 2):

L61, Y62, F67, K106, G145, I212, S151, R214, Y150, F143, R146, L241, I236, L7, V259, F284, F350, F343, L427 and/or V481, in particular at least one of the following mutations:

L61W,V,F;

Y62W;

F67W;

K106R,F,W;

G145F,W

I212F,L,W,Y,R,K;

S151 replaced with any other amino acid residue and in particular with F,W,I or L;

R214W;

Y150R,K;

F143W;

R146W;

L241I,F,Y,W;

I236L,F,W,Y;

L7F,I,W;

V259F,I,L;

F284W;

F350W;

F343W;

L427F,L,W; and/or

V481,F,I,L,W.

49. A variant of a parent Termamyl-like α-amylase, which exhibits α-amylase activity and which has a reduced capability of cleaving an oligo-saccharide substrate close to the branching point as compared to the parent α-amylase, which variant comprises a mutation of an amino

acid residue corresponding to at least one of the following positions of the *B. licheniformis* α-amylase (SEQ ID NO: 2):

V54, D53, Y56, Q333 and/or G57, in particular at least one of the following mutations:

V54L,I,F,Y,W,R,K,H,E,Q;

D53L,I,F,Y,W;

Y,56W;

Q333W; and/or

G57 to all possible amino acid residues.

50. The variant according to claim 17, wherein one or more proline residues present in the amino acid residues with which the parent α-amylase is modified are replaced with a non-proline residue such as alanine.

51. The variant according to claim 17, wherein one or more cysteine residues present in the amino acid residues with which the parent α-amylase is modified are replaced with a non-cysteine residue such as alanine.

52. A DNA construct comprising a DNA sequence encoding an α-amylase variant according to claim 17.

53. A recombinant expression vector which carries a DNA construct according to Claim 52.

54. A cell which is transformed with a DNA construct according to Claim 52.

55. A cell according to Claim 54, which is a microorganism.

56. A cell according to Claim 55, which is a bacterium or a fungus.

57. The cell according to Claim 56, which is a grampositive bacterium such as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus lentus*, *Bacillus brevis*, *Bacillus stearothermophilus*, *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus coagulans*, *Bacillus circulans*, *Bacillus lautus* or *Bacillus thuringiensis*.

58. Use of an α-amylase variant according to claim 17 for washing and/or dishwashing.

59. Use of an α-amylase variant according to claim 17 for desizing.

60. Use of an α-amylase variant according to claim 17 for starch liquefaction.

61. A detergent additive comprising an α-amylase variant according to claim 17, optionally in the form of a non-dusting granulate, stabilised liquid or protected enzyme.

62. A detergent additive according to Claim 61 which contains 0.02-200 mg of enzyme protein/g of the additive.

63. A detergent additive according to Claim 61, which additionally comprises another enzyme such as a protease, a lipase, a peroxidase, another amylolytic enzyme and/or a cellulase.

64. A detergent composition comprising a surfactant and an α-amylase variant according to claim 17.

65. A detergent composition according to Claim 64 which additionally comprises another enzyme such as a protease, a lipase, a peroxidase, another amylolytic enzyme and/or a cellulase.

66. A manual or automatic dishwashing detergent composition comprising an α-amylase variant according to claim 17.

67. A dishwashing detergent composition according to Claim 66 which additionally comprises another enzyme such as a protease, a lipase, a peroxidase, another amylolytic enzyme and/or a cellulase.

68. A manual or automatic laundry washing composition comprising an α-amylase variant according to claim 17.

69. A laundry ~~washing composition according to Claim 68,~~ which additionally comprises another enzyme such as a protease, a lipase, a peroxidase, an amylolytic enzyme and/or a cellulase.

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